IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Gladwin *et al.*
Application No. 10/563,683
Filed: October 4, 2006
Confirmation No. 3225
For: USE OF NITRITE SALTS FOR THE TREATMENT OF CARDIOVASCULAR CONDITIONS
Examiner: Anna Pagonakis
Art Unit: 1628
Attorney Reference No. 4239-67618-07

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DECLARATION UNDER 37 C.F.R. § 1.132

1. I, Mark T. Gladwin, M.D., am a co-inventor named in the above-referenced patent application. I am Chief of the Pulmonary, Allergy and Critical Care Medicine Division at the University of Pittsburgh School of Medicine in Pittsburgh, Pennsylvania. I have conducted research in the field of pulmonary disease for over 12 years and I have published more than 180 peer-reviewed scientific articles. A copy of my current *curriculum vitae* is submitted herewith as Exhibit A.

2. It is my understanding that in the Office action dated July 27, 2010, claim 4 is rejected as allegedly obvious in view of Shaw *et al.* (U.S Patent No. 4,650,484), Goldfrank *et al.* (Goldfrank's Toxicological Emergencies, 7th Edition, 2002, page 1511) and Modin *et al.* (*Acta. Physiol. Scand.* 171:9-16, 2001). It is also my understanding that the Office alleges that the results obtained in Modin *et al.* using aortic ring bioassays are representative of results that would be obtained *in vivo*. I disagree.

3. The aortic ring bioassays of Modin *et al.* were conducted in the absence of red blood cells and hemoglobin, both of which inhibit vasodilation induced by nitric oxide (NO) and acetylcholine. In fact, red blood cells and hemoglobin have previously been used in aortic ring

bioassays specifically to inhibit vasodilation induced by NO, NO donors and acetylcholine (Fujiwara *et al.*, *J Neurosurg* 64:445-452, 1986). Exhibit B includes FIG. 5A from Fujiwara *et al.* which illustrates that as little as 1 μM hemoglobin inhibits NO-induced vasodilation in a ring bioassay. Because of the inhibitory effect of red blood cells and hemoglobin on NO-induced vasodilation, a researcher (including myself) would have expected that in an *in vivo* setting, where both red blood cells and hemoglobin are present, nitrite (which is converted to NO) would have decreased potency relative to the potency of nitrite in an aortic ring bioassay performed in the absence of red blood cells and hemoglobin.

4. Accordingly, a researcher would not expect that the results obtained using an aortic ring bioassay in the absence of red blood cells and hemoglobin to measure the vasodilatory activity of nitrite would be representative of the results obtained *in vivo*. Rather, one would have expected that the concentration of nitrite required to induce vasodilation *in vivo* would have been greater than the concentration required to achieve the same effect in the aortic ring bioassay.

5. I was asked to review the four references cited in the Office action dated July 27, 2010 (U.S. Patent No. 6,153,186; U.S. Patent No. 5,436,271; U.S. Patent No. 6,110,453; and Gladwin *et al.*, *Free Radic Biol Med* 36(6):707-717, 2004). I understand that the Office alleges that these references teach that results obtained using aortic ring bioassays are also obtained in an *in vivo* environment.

6. None of the references cited in the July 27, 2010 Office action teach that vasodilatory concentrations of nitrite or NO *in vivo* correlate with vasodilatory concentrations in an aortic ring bioassay. Based upon the cited references and the general knowledge available in the field as of the priority date of the current application, one of skill in the field would not have concluded that the concentration of sodium nitrite identified by Modin *et al.* as inducing relaxation of the aortic segment in an aortic ring bioassay would be the same concentration of sodium nitrite that causes vasodilation *in vivo*. Our results were extremely unexpected and highly controversial in the field when they were first published in *Nature Medicine* (Cosby *et al.*, *Nat Med* 10(10):1122-1127, 2003; of record). Debate over our findings was intense for two years until our original vasodilation studies were confirmed in animal models.

7. Gladwin *et al.*, a review article I co-authored, describes the finding that hypoxia and acidosis potentiate NO generation and vasodilation from nitrite and other NO donors in an aortic ring assay (page 711, column 1, first paragraph). The review article also states that possible mechanisms for the *in vivo* conversion of nitrite to NO include enzymatic reduction by xanthine oxidoreductase and nonenzymatic disproportionation/acidic reduction, both of which would preferentially occur in vascular regions with low pH and low partial pressure of oxygen (page 710, second column). This manuscript does not teach that *in vivo* vasodilatory concentrations of NO or nitrite correlate with vasodilatory concentrations of NO or nitrite in an aortic ring bioassay.

8. In regard to U.S. Patent No. 6,153,186, the Office points to column 7, lines 64-65, which recites "The vessel ring bioassay data of FIG. 4A agree well with the in vivo data of FIG. 5." However, the results shown in FIG. 4A and FIG. 5 are not for experiments that measure the vasodilatory activity of nitrite. Rather, these experiments were performed to evaluate the effect of Hb(FeII)O_2 and SNO-Hb(FeIII) (a synthetically prepared NO donor molecule of the S-nitrosothiol class) on blood pressure.

9. U.S. Patent No. 5,463,271 describes the use of rat aortic rings to test the inhibitory effect of N^6-(hydrazinoiminomethyl)-L-lysine on nitric oxide synthesis *in vitro*. Nitrite is used in this study as a biomarker of NO synthesis rate (NO being oxidized to nitrite). This patent does not compare the results obtained in the *in vitro* aortic ring assay to an *in vivo* system and therefore provides no evidence that the data obtained using an aortic ring assay is comparable to data that would be obtained *in vivo*. Furthermore, this patent does not provide any teachings related to the vasodilatory activity of nitrite *in vivo* or *in vitro*.

10. U.S. Patent No. 6,110,453 describes an experiment to test vascular relaxation caused by a polymer-bound nitric oxide-releasing composition in an aortic ring bioassay. The compound described in this patent is an NO-releasing molecule, but much like other NO donors, is not at all the same as the anion nitrite. NO donors are known to be inhibited by hemoglobin in

aortic ring preparations (see paragraph 3 above). Moreover, this patent does not compare the results obtained using the aortic ring assay to an *in vivo* system.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12-20-2010, 2010

Mark T. Gladwin, M.D.